Supporting Information

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Materials and Methods

PCR Array. Total RNA samples were isolated from frozen tissues using TRIzol (Invitrogen). Gene expression levels in EB-high and EB-low areas [after i.v. LLC tumor-conditioned medium (LTCM) and recombinant VEGF infusion] were measured using Mouse Endothelial Cell Biology PCR array plates (SABiosciences).

Immunoprecipitation and Western Blot Analysis. Lung tissues were lysed with lysis buffer [50 mM Hepes (pH 7.4), 1% Triton X-100, 150 mM sodium chloride, and 1 mM EGTA and 5 mM EDTA] with protease inhibitor mixture, 1 mM phenylmethylsulfonyl fluoride, 1 mM sodium fluoride, and 1 mM sodium orthovanadate. For FRNK detection, we used the rabbit antibody to the carboxyl terminal FAK (C-20; Santa Cruz Biotechnology).

Isolation of Endothelial Cells. Minced mouse lungs were digested with 2 mg/mL of collagenase type 1A, 2 mg/mL of hyaluronidase, and DNase at 37 °C for 30 min, and then filtered through a 70-μm cell strainer (all from Sigma). The cellular filtrate was washed, and a layer containing endothelial cells was separated using Histopaque (Sigma). Cells from this layer were incubated with magnetic Dynabeads (Dynal) coated with anti-rat IgG antibody, to which rat anti-mouse CD31 and MECA32 antibodies were attached. Cells were separated using a magnetic separator and then cultured on a collagen-coated plate in VEGF-free EBM2 medium (Lonza) overnight.

Soluble Protein Measurement in Cell Culture Supernatant. We measured mouse VEGF and mouse placental growth factor levels using the ELISA plate from R&D Systems, according to the manufacturer's instructions.

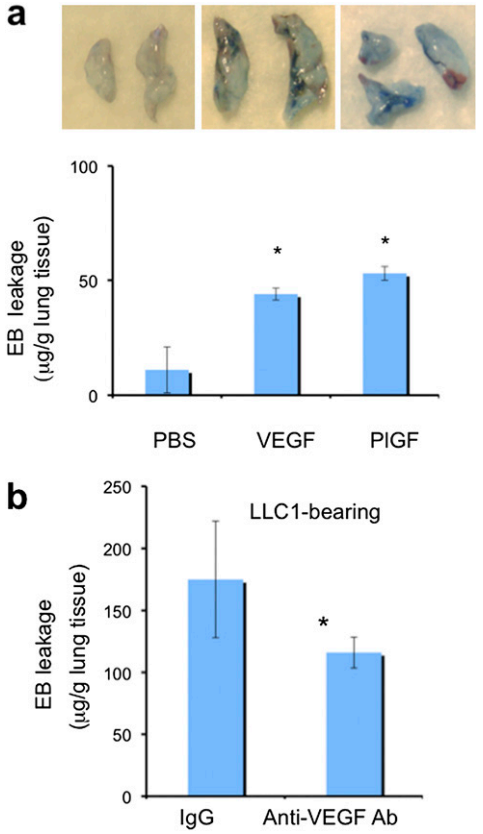

Fig. S1. (A) *Upper:* Distinct macroscopic regions of Evans Blue (EB) leakage in the lungs of healthy mice after stimulation by recombinant (r)VEGF or recombinant placental growth factor (rPlGF). *Lower:* Total EB leakage into lungs of healthy mice 3 h after stimulation with rVEGF or rPlGF. *$P < 0.05$, $n = 6$ and $n = 4$, respectively. (*B*) Total EB leakage into the lungs of mice bearing LLC primary tumors measured 3.5 h after EB injection, with or without treatment with an anti-VEGF antibody. $n = 4$, ± SEM.

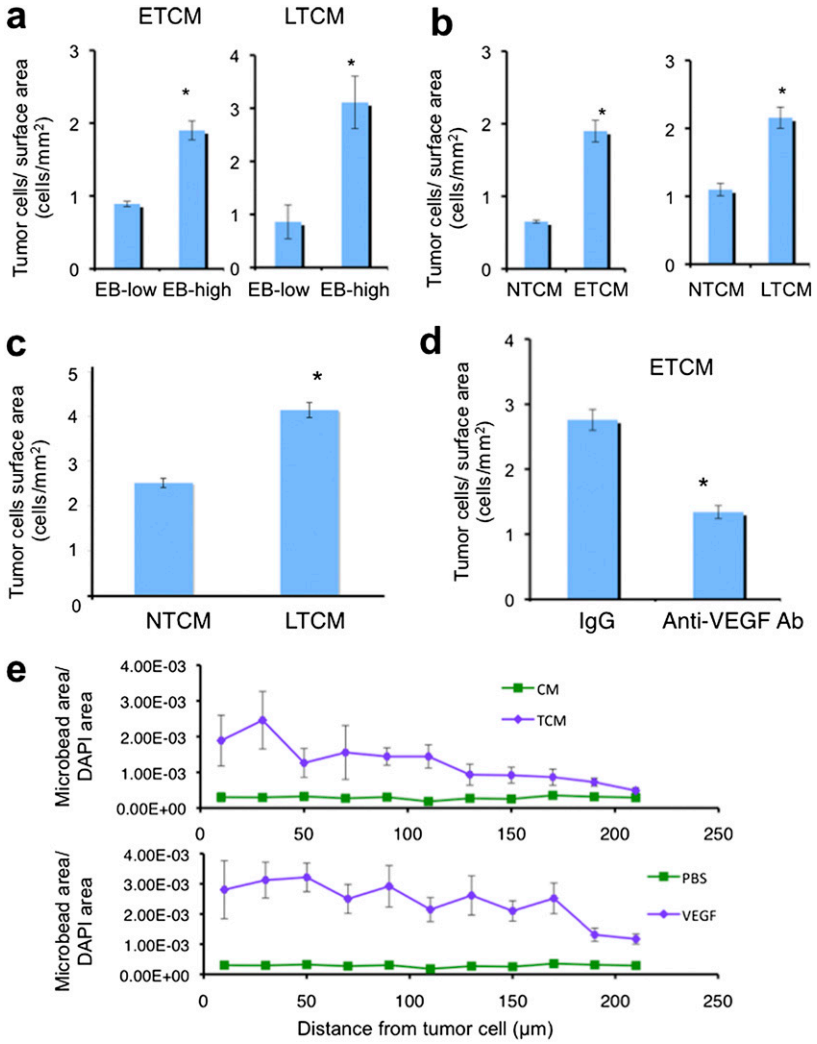

Fig. S2. (Continued)

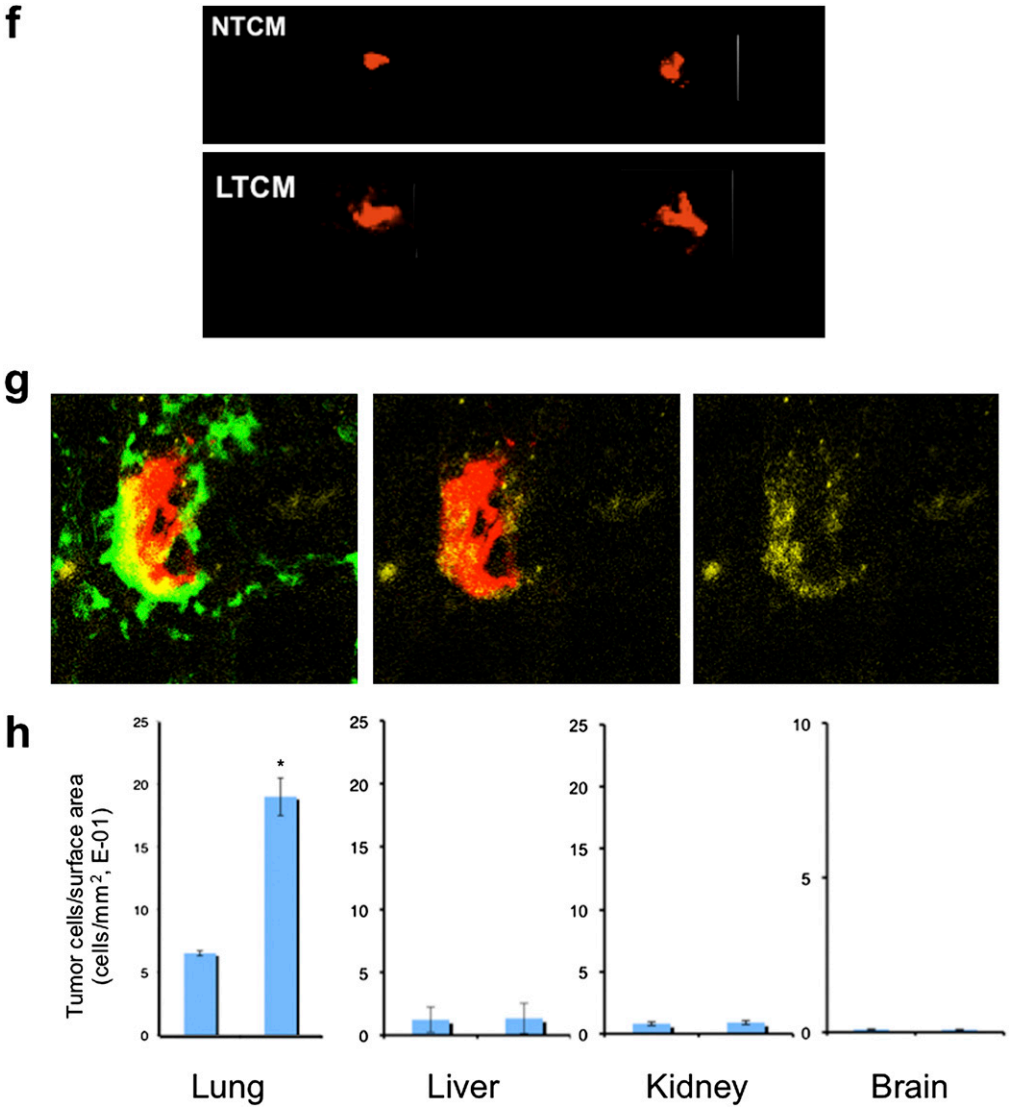

Fig. S2. (*A*) Number of i.v. injected metastatic cancer cells homed to areas of high vs. low Evans Blue (EB) leakage in tumor-bearing mice measured 5 h after injection; $n = 4$, $^*P < 0.05$, ± SEM. (*B*) Tumor cell homing to lungs stimulated by E0771 tumor-conditioned medium (ETCM) or LLC TCM (LTCM) 5 h after tumor cell injection. $^*P < 0.05$, $n = 6$. (*C*) Tumor cell (LLC) homing to the lungs of mice stimulated by LTCM, measured 24 h after tumor cell injection. $^*P < 0.05$, $n = 6$. NTCM, non–tumor-conditioned medium. (*D*) Number of E0771 metastatic cancer cells homed to the lungs of ETCM-stimulated mice measured 5 h after tumor cell injection, with or without anti-VEGF antibody treatment; $n = 4$, $^*P < 0.05$. (*E*) Density of PEG microbeads (*y*-axis) measured against distance from tumor cell (*x*-axis) in the lungs of mice stimulated by NTCM, TCM, VEGF, and PBS (*y*-axis units are in multiples of 10^{-3}). (*F*) Representative images of tumor cells in the lungs of wild-type mice after stimulation with NTCM or TCM (24 h after stimulation). (*G*) Representative image of an extravasated LLC cell 24 h after i.v. injection (red, tumor cell; yellow, PEG microbeads; green, lectin-stained vessels). (*H*) LLC tumor cell homing to lungs, liver, kidney, and brain after stimulation with i.v. PBS (left bars) or TCM (right bars). $^*P < 0.05$; $n = 6$ mice per group.

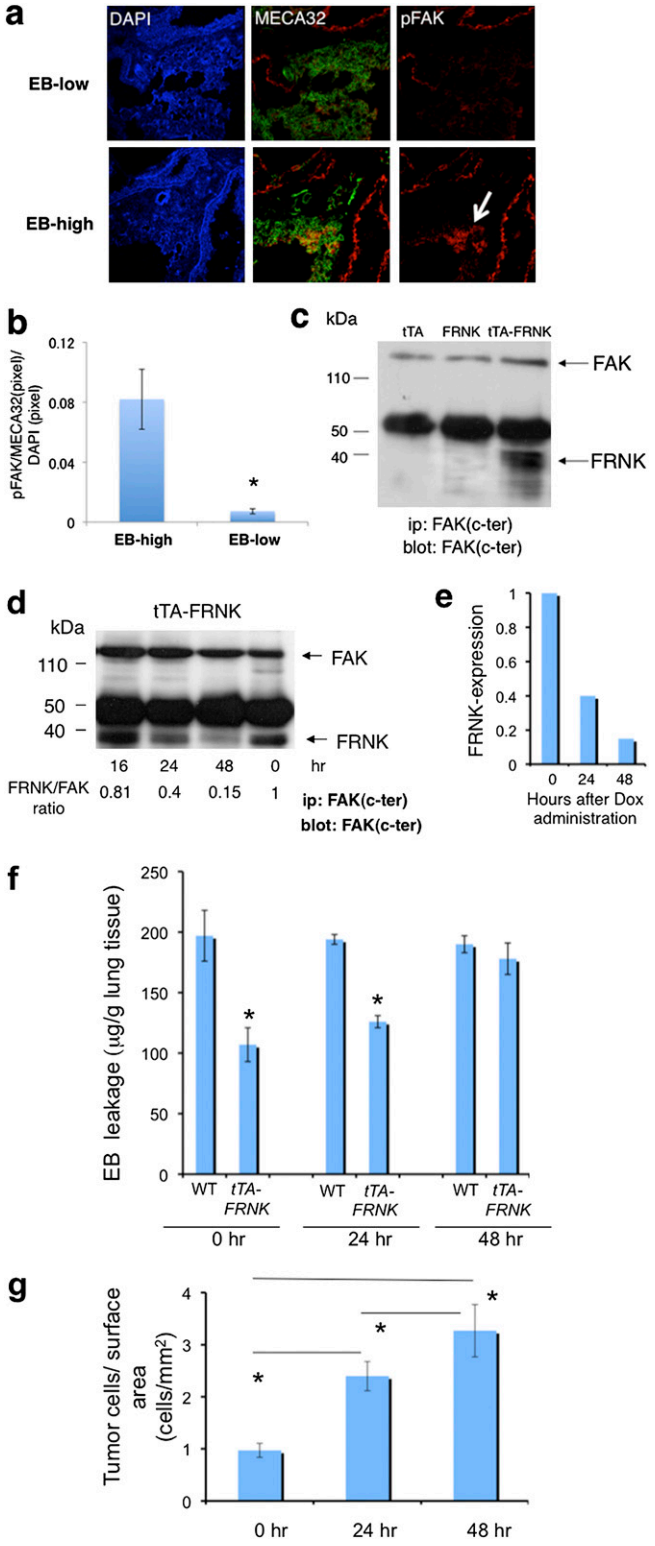

Fig. S3. (*A*) Representative images showing immunofluorescent staining for phosphorylated FAK (Y397) in the Evans Blue (EB)-low vs. EB-high areas of the lung after stimulation by E0771 tumor-conditioned medium (ETCM). Staining is by DAPI (blue), anti-MECA32 (endothelial cell marker, green), and anti pFAK (red). *Center:* Merged image. (*B*) Quantification of pFAK and mECA32 colocalization by double immunostaining. (*C*) Western Blot showing expression of FAK and FRNK proteins in tTA (control), FRNK (control), and *tTA-FRNK* mice (in the absence of doxycycline). (*D*) Kinetics of FRNK protein suppression after i.p. doxycycline injection in *tTA-FRNK* mice. (*E*) FRNK expression as a ratio of baseline FAK expression at different time points after i.p. doxycycline. (*F*) EB leakage in ETCM-stimulated lungs from wild-type and *tTA-FRNK* mice at 0, 24, and 48 h after i.p. injection of doxycycline. $*P < 0.05$, $n = 4$. (*G*) Number of E0771 cells homing in ETCM-stimulated lungs from *tTA-FRNK* mice 0, 24, and 48 h after i.p. injection of doxycycline. $*P < 0.05$, $n = 4$.

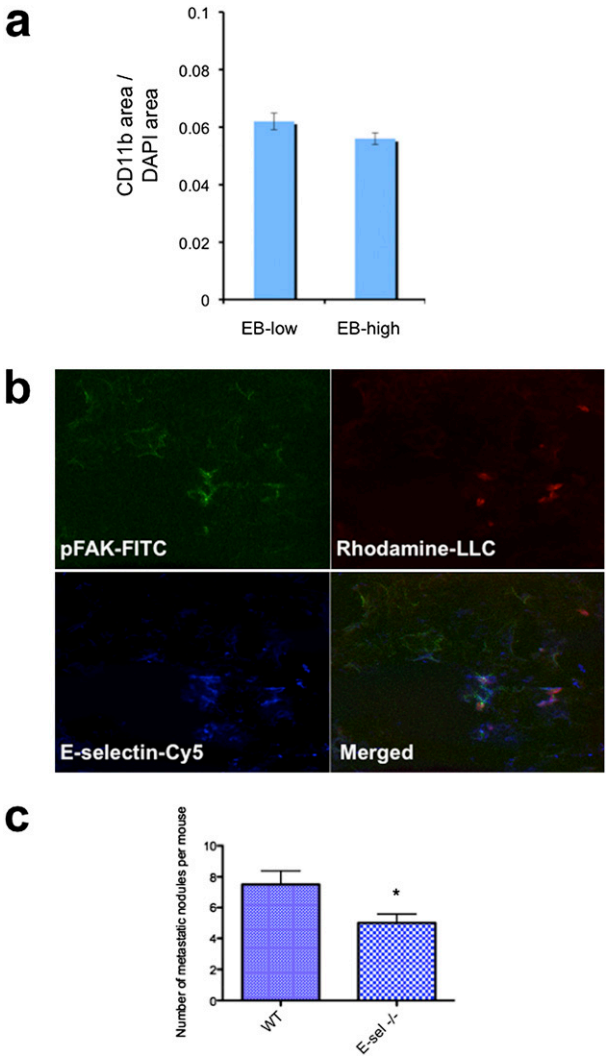

Fig. S4. (*A*) Immunohistochemical quantification of CD11b myeloid cells in the tumor-conditioned medium (TCM)-stimulated lungs of wild-type mice (3.5 h after i.v. TCM injection); $n = 4$. (*B*) Representative immunohistochemical image depicting colocalization of phosphorylated FAK expression (green), E-selectin expression (blue), and tumor cell homing (red). (*C*) Metastatic burden in mice after i.v. infusion of 200 μL of LLC TCM followed by i.v. infusion of 50,000 LLC cells after 3.5 h: E-selectin deficiency reduces the number of lung metastases formed after 20 d. $*P < 0.05$, $n = 6$.